Journal of Applied and Natural Science 3 (2) : 258 -263 (2011)

JANS

Chromium (III) induced haematological alterations in Indian common carp, *Labeo rohita* (Ham.)

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Abstract: The principle source of chromium in water bodies is discharged from industries which use large amount of chromates or dichromates. The freshwater fish, *Labeo rohita* was used as a model in the present studies to investigate the responses to chromium metal salt contamination in water bodies as it is the most common fish consumed widely in India. Haematological tests were carried out as these are the important diagnostic tools and are equally valuable as indicators of disease or stress due to pollutants and environmental fluctuations. The fish *Labeo rohita* were exposed to sublethal concentration of chromium chloride for 10, 20 and 30 days to study alterations in certain haematological parameters. The parameters under study were total erythrocyte count (TEC), haemoglobin (Hb gm%), total leucocyte count (TLC), differential leucocyte count (DLC) and pack cell volume (PCV). The studies revealed that chromium chloride-treated *Labeo rohita* exhibited decreased levels of total erythrocyte count (TEC), haemoglobinn (Hb gm%) and PCV whereas, total leucocyte count (TLC) was increased under the stress of chromium metal The decreased WBC count found after 30 days along with depleted Hb content and RBC count indicate dysfunctioning of haemopoietic systems along with dysleucopoiesis. Leucocytosis observed after 10 and 20 days exposure has been considered to be an adaptation to meet stressful conditions by animals. Increase in macrophages and basophils appears to be protective response during chromium exposure. An increasing trend in the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were evident in fish exposed to sublethal doses of chromium chloride when compared with the control.

Keywords: *Labeo rohita*, Pollution, Chromium, Fish, Haematology

INTRODUCTION

The pollution of freshwaters is probably the single most significant factor in causing major declines in the population of many fish species in India and elsewhere. In context to the heavy metal toxicity two major aspects, firstly, the use of fish as an indicator organism for the heavy metal pollution of its environment and its possible unfitness for human consumption from a toxicological point of view and secondly, the use of fish to study the physiological behaviour of heavy metal and effect of heavy metals on the metabolism of the fish are generally considered in investigations.

Good quality of food for consumption can only be produced in an environment free from contamination. Fish, having great economic importance, are affected immensely by various chemicals including heavy metals directly or indirectly in various ways. Several reports indicate high mortality of juvenile fish and reduced breeding potentiality of adults after long term exposure to heavy metals **(**McLeay, 1975**)**. The freshwater fish, *Labeo rohita* is of great commercial importance because it is the most common fish consumed widely worldwide. Therefore, it can be a good model to study the responses to heavy metal contamination. Chromium (III) is a major contaminant of water bodies. It is highly lethal for the organisms because of its ability to accumulate in the tissues of organisms. The principle contamination of chromium results from discharges from textile industries using large amount of chromates or dichromates compounds. Chromium is also used in chromium plating, steel fabrication, paints, pigment manufacturing and leather tanning industries.

Haematological tests are important diagnostic tools and valuable as indicators of disease or stress due to pollutants and environmental fluctuations (Calabrese *et al.*, 1975). The blood also plays an integrated and inevitable part in the immune systems (Hymavathy and Rao, 2000, Kori-Siakpere and Ubogu, 2008) and therefore can be used to ascertain the healthy state of fish when exposed to pollutants. Piscine haematology is useful in assessing the health and general condition of the animals subjected to the changing environmental conditions. Effects of heavy metals on haematological parameters have been studied by many workers (Singh, 1995; Nanda

ISSN : 0974-9411(Print), 2231-5209 (Online)

and Behera, 1996; Iqbal *et al.*, 1997; Ray and Banerjee, 1998; Hymavathi and Rao, 2000; Vijayamohan *et al.*, 2000). Long-term effects of heavy metal on blood chemistry parameters have been established by Gardner and Yeuich (1969). As the reports on the effect of chromium on the haematological parameters of the fishes are sparse, so it was decided to undertake the present study.

MATERIALS AND METHODS

Disease-free fish, *L. rohita* were bathed in 1% $KMnO_4$ solution and acclimated in big glass aquarium of 400 to 450 liter capacity for a period of 15 days. Chlorine free aged tap water was used in the aquaria. The water was maintained at pH 8.2 ± 0.2; hardness 280 mg/l; D.O. 6.2 mg/l; total alkalinity 310 mg/l and temperature of 25 ± 2^0C. The fish were fed with rice bran daily at 10.30 am. The water in the aquaria was changed daily after the consumption of food supplied. The healthy fish of both the sexes having uniform size and weight (125 ± 2g) were selected from the lot for experimental purpose. Initially 96h LC_{50} doses were determined for chromium metal compound by the method as described in standard methods by the APHA (1998).

Forty healthy fish from the stock were selected and were divided into two groups. Group-I consisted of 20 fish in aged tap water which served as control whereas Group-II consisted of 20 fish kept in toxicant water containing 6mg/l chromium chloride ($1/10^{th}$ of its 96h LC_{50} concentration) for 30 days. To avoid the effects of starvation, the fish were fed on the rice bran at the average feeding rate of 25mg food / gm fish / day. The toxicant solutions and the aged tap water (control) were renewed every day in the morning after removing the unused food to maintain uniform test concentration throughout the experimental period. The controls as well as experimental fish were sacrificed on the 10, 20 and 30 days of exposure to chromium chloride. The blood was collected into vials containing heparin as anticoagulant by severing the caudal peduncle.

Haemoglobin (Hb%) was measured by Sahli's haemometer. RBC (TEC) and WBC (TLC) were counted by Neubaur's haemocytometer using Hayem's and Turk's solutions as diluting fluids, respectively. Packed cell volume (PCV) was measured by Wintrobe's method (300 rpm for 1 h). Differential leucocyte count (DLC) was carried out by preparing a thin blood smear and staining it with Leishman's stain. Calculation of mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean cell volume (MCV) was done using standard formulae (Dacie and Lewis, 1982). The statistical data analysis was done by the student's t-test.

RESULTS

The results of exposure of fish, *Labeo rohita* to sublethal concentration of chromium chloride are shown in Table 1-3. The stress of chromium metal caused the decreased in haematological parameters like total RBC, Hb gm%, and PCV whereas, total WBC count was increased.

Chromium chloride caused uniform reduction in the RBC count and Hb content of the blood, following all the periods of exposure of the experimental fish (Table 1). The slight improvement was seen in TEC and Hb gm% after 30 days of treatment. The percent decrease in TEC after 20 days was 22.26% however after 30 days this decrease was only 12.98% (Table, 1). The results of haemoglobin contents also showed slight improvement after 20 days of treatment as the decline in Hb% was 26.57 after 20 days and 23.26 after 30 days of exposure (Table 1).

The significant increase was observed in total WBC count of fish after treatment with chromium metal salt for 10 ($p< 0.001$) and 20 days exposure ($p< 0.01$). After 30 days of exposure, 7.72% depletion was recorded in total WBC count of chromium chloride treated fishes which was not significant (Table 1). Declined PVC under the stress of heavy metal salt chromium chloride was found to be significant ($p < 0.01$) after 30 days of exposure. (Table 1). Blood parameters like MCV, MCH and MCHC were increased with the increase in the exposure period of chromium chloride (Table 2). The results of differential leucocyte counts (DLC) are presented in Fig. 1. Per cent population of Macrophages, Eosinophils and Basophils was found to be increased in the experimental fishes. But at the same time the percent population of lymphocytes and monocytes was decreased. Further the increase in macrophages was found to be duration dependent.

DISCUSSION

The present study clearly indicates that exposure of *L. rohita* to sublethal concentration of chromium chloride elicited unequivocal changes in blood parameters.

The reduction in RBC count and Hb content after 20 days of exposure indicates anaemia associated with erythropenia. Our results are similar to the earlier reports of Panigrahi and Mishra (1978), Agarwal *et al.* (1983) and Kori- Siakpere and Ubogu *et al.* (2008). Anaemia with erythropenia has also been reported earlier in fishes after exposure to sublethal doses of other metals; mercury, lead and zinc (Srivastava and Mishra, 1979; Goel and Gupta, 1985). It was also reported that decrease in TEC and Hb content might also be due to failure of renal haemopoiesis as the kidney was found to be damaged due to exposure of the fish to heavy metals (Gliess, 1983; Das *et al.*, 1988). Further various authors have reported accumulation of heavy metals like zinc, copper, nickel and chromium in the kidney of fishes (Rana and Raizada, 2000; Khunyakari *et al.*, 2001; Dhanapakian and Ramaswamy, 2001). Accumulation of chromium might have

Table 1. Total erythrocyte count (TEC), Haemoglobin (g%), Total leucocyte count (TLC), and Packed cell volume (PCV) in the fish, *Labeo rohita* exposed to sublethal concentration of heavy metal salt, Chromium chloride.

Days of exposure	Total erythrocyte count TEC (million/mm^3)		Haemoglobin (g%)		Total leucocyte count (10^3cells/mm^3)		Packed cell volume (PCV) (ml/100ml)	
	Control	Chromium chloride	Control	Chromium chloride	Control	Chromium chloride	Control	Chromium chloride
10	2.72$\pm$0.28	2.32$\pm$0.71 (-14.71) p<0.05	5.60$\pm$0.05	5.20$\pm$0.24 (-7.14) NS	6.27$\pm$0.36	7.72$\pm$0.17 (+23.12) p<0.001	50.14$\pm$1.24	46.72$\pm$1.17 (-6.82) NS
20	2.65$\pm$0.19	2.06$\pm$0.80 (-22.26) p<0.05	5.72$\pm$0.16	4.20$\pm$0.12 (-26.57) p<0.01	6.30$\pm$0.23	8.17$\pm$0.27 (+29.68) p<0.01	50.07$\pm$1.20	44.27$\pm$1.20 (-11.58) p<0.05
30	2.62$\pm$0.19	2.28$\pm$0.28 (-12.98) p<0.01	5.16$\pm$0.5	3.96$\pm$0.29 (-23.26) p<0.001	6.35$\pm$0.28	5.86$\pm$0.16 (-7.72) NS	49.80$\pm$1.19	28.93$\pm$0.88 (-41.91) p<0.01

also affected renal haemopoiesis in the present investigation. Christy (1995) had reported damaged RBC in the fish *Catla catla* exposed to potassium dichromate. Later Adeyemo (2007) also reported deformed RBC in the fish *Clarias gariepinus*. Impairment in the uptake and absorption of iron might be the one of the reasons behind the lower Hb content in the experimental fishes exposed to sublethal doses of the heavy metal salts. It seems that in the present investigation various changes in the blood parameters of *L. rohita* such as damage to chief haemopoietic organs, increased destruction of circulating RBC and impaired intestinal absorption of iron might have collectively resulted in the lower Hb content (Kothari and Saxena 1997).

Nanda and Behera (1996) reported decrease in total RBC, Hb% and PCV in the fish, *Heteropneustes fossilis* after nickel sulphate treatment for 15 days. Haemolysis of erythrocytes was also observed after exposing the erythrocytes, experimentally *in vitro*, to 2mM dichromate for 24 hours (Roche and Boget, 1993). in and recently studied reported the Haematological effects of hexavalent chromium were studied in fresh water teleost, *Oreochromis mossambicus* (Ali *et al.*, 2000) and in Indian Major Carp, *L. rohita* (Vutukuru, 2005) and similar results with reduction in Hb, TEC and Hct values were reported.The present investigation revealed experimental fishes exposed to chromium chloride, exhibited erythrocytosis and increase in Hb content following 30 days of stress after initial decrease (Tables 1 and 2). This can be due to impairment of gas exchange by the gills and lining of operculum (Grizzle, 1977; Larson *et al.*, 1980; Haniffa and Porchelvi, 1985, Osman *et.al.*, 2009; Bhatkar, 2010) and the consequent excitation or stimulation of erythropoiesis or compensatory erythropioesis **(**Larson *et al.*, 1980; Shah, 2006; Kori-Siakpere and Ubogu, 2008; Adeyemo, 2007**)**. However this type of compensatory reaction usually stimulates erythropioesis, thereby leading to the release of immature erythrocytes into the circulating blood (Kori-Siakpere and Ubogu, 2008).

In the experimental fishes the PCV values decreased significantly due to decline in RBC count. Appreciable decline in PCV in the present study reflects the anaemic state of fish. Vutukuru (2005) reported the similar findings in *L. rohita* on exposure to hexavalent chromium. In the opinion of Johansson-Sjobeck and Larsson (1979) anaemia is an early manifestation of acute and chronic intoxication of heavy metals. Importance of these changes may be understood in terms of oxygen consumption in fish resulting in mass fish kills due to heavy metal pollution. Like other stressors and pollutants (Banerjee, 1986), heavy metals influenced and increased red cell indices. Reasons that may be attributed for the anaemic state of fish under the toxic stress of chromium chloride may be inhibition of erythropioesis and haemopoietic tissue. Under the stress condition fish exhibits asphyxiation due to respiratory failure and anaerobic glycolysis is enhanced. Thus, these physiological changes suggest prevalence of hypoxic environment in the blood of the treated fishes, which cause haemodilution (Larsson, 1975).

Further, Singh (1995) has reported the significant decrease in TEC, hemoglobin per cent, MCH and hematocrit in *Channa punctatus* exposed to both copper and chromium and this decrease is more pronounced in fishes exposed to chromium suggesting that the metal induces acute anemia under toxic conditions. In the present study, the anemia could be probably due to structural alterations of heme leading to disturbed hemoglobin synthesis and also the inhibitory effect of chromium on the enzyme system in the synthesis of hemoglobin cannot be ruled out as suggested in earlier studies (Johansson-Sjobeck and Larrsson, 1979).

Increase in WBC count as in the present study was also reported by Murugesan and Haniffa (1985) in heavy metal-treated fish blood, suggesting induction of some pathology and also this might be due to the effect of

Table 2. Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), and Mean corpuscular haemoglobin concentration (MCHC) in the fish, *Labeo rohita* exposed to sublethal concentration of heavy metal salt, Chromium chloride.

Days of exposure	Mean corpuscular volume (MCV) (cu.µ)		Mean corpuscular haemoglobin (MCH) (pg)		Mean corpuscular haemoglobin concentration (MCHC) (%)	
	Control	Chromium chloride	Control	Chromium chloride	Control	Chromium chloride
10	136.36±6.12	145.96 ±11.85 (+7.04) NS	30.26 ±2.02	50.23±1.26 (+65.99) p<0.001	26.46±1.26	33.76±1.82 (+27.59) p<0.01
20	132.07±7.62	152.01±9.85 (+15.10) NS	32.03±1.26	53.00±1.95 (+65.47) p<0.001	24.79±1.85	39.26±3.76 (+58.37) p<0.001
30	128.62±8.25	150.00±8.65 (+16.62) p<0.01	32.06±1.24	75.66±3.65 (+135.99) p<0.001	24.55±1.6	63.92±4.24 (+160.37) p<0.001

The results are represented as average of five observation ±SE, Fig. in paranthesis indicate increase or decrease of control.

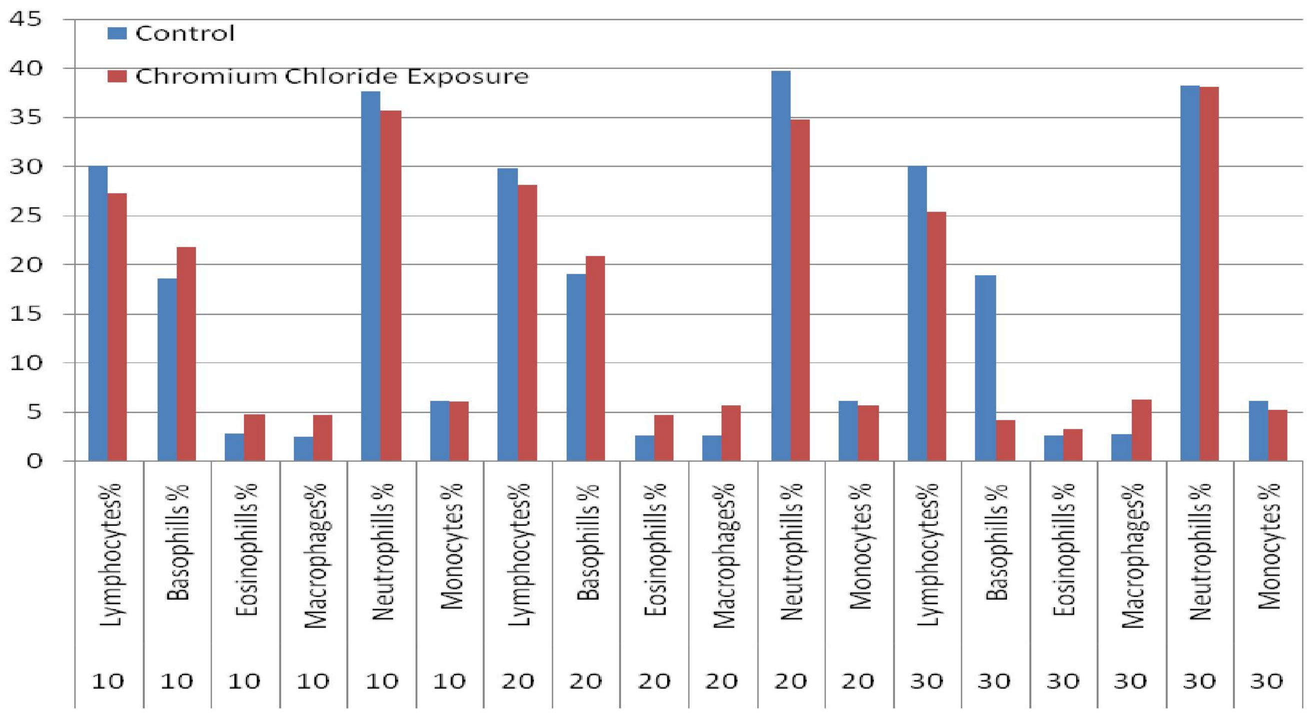

Fig.1. *Differential leucocyte count (DLC) in the fish, Labeo rohita exposed to sublethal concentrations of heavy metal salts, Chromium chloride.*

metal toxicants on bone marrow. Leucocytosis has been considered to be an adaptation to meet stressful conditions by animals. This is in agreement with the finding of Garg *et al.* (1989) and Hota (1995). In the present investigation depletion in WBC count was observed after 30 days of exposure. The depleted WBC count along with depleted Hb content and RBC count indicates dysfunctioning of haemopoietic systems along with dysleucopoisis. This is most probably due to bone marrow depression and liver dysfunction (António *et al.*, 2007; Osman *et al.*, 2009).

According to the study conducted by Gill and Pant (1987) in fresh water fish *Barbus conchonius*, the leucocytes showed initial rise (leucocytosis), which was followed by leucopenia. This decrease in chromium-exposed fish, according to the report, was due to decrease in neutrophils. Srivastava *et al.* (1979) demonstrated that the exposure of the teleost fish to sublethal dose of chromium (35 ppm), was responsible for decrease in the leucocytes, which in turn was due to reduction in numbers of small lymphocytes.

The increase in leucocyte number in all the experimental fishes after 10 and 20 days of heavy metal treatment is probably for the removal of cellular debris of necrosed tissue at quicker rate as reported by McLeay and Brown (1974) in *Oncorhynchus kisutch* under the chemical stress. Several other authors also reported an increase in leucocyte count in various fresh water fishes exposed to different heavy metal salts which supports findings in the present study (Agrawal and Srivastava, 1980; Singh, 1995; Ray and Banerjee, 1998; Ali *et al.,* 2000). The increase in number of WBCs may play an important role in immunological defense systems during exposure to toxicants like heavy metals and appears to be associated with increased circulatory levels of granulocytes, which are known to respond for phagocytosis (Briton, 1963; Kori-Siakpere and Ubogu, 2008). This suggests the development of a certain degree of tolerance during toxicant stress condition.

An increasing trend in the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were evident in fish kept in sublethal doses of chromium chloride when compared with the control. These haematological disturbances are a haemopoietic or erythrocyte mobilization response to hypoxemia induced by heavy metal stress. The values of MCV and MCH were found to increase during macrocyte anaemia (Bomford *et al*., 1975). Wepener *et al.,* (1992) studied the effect of hexavalent chromium on the haematology of *Tilapia sparrmanii* and similar results, which are well in agreement with the present results. This type of macrocytic anaemia may reflect possible chronic liver damage (Dacie and Lewis, 1977).

In all the experimental fishes increase in the macrophages was observed. The increased macrophages could engulf the cell debris and could eliminate them preventing bacterial infection in the damaged tissues. Thus increased population of macrophages is a bio-indicator of tissue damage. The parameter, DLC, appears to be neglected in fish studies as very few reports are available showing DLC in heavy metal exposed fishes (Briton, 1963; McLeay, 1975; Goel *et al*., 1984; Thakur and Pandey, 1990). Generally, in mammals, an increase in lymphocytes is seen when the tissues like liver, lung and kidney get damaged due to heavy metal toxicants or pesticides or drugs or any other toxic substance or germs. Its increase is correlated with the immunological reaction as these cells secrete the globulin proteins like α, β and γ globulins, which participate in immune reactions. However, in fishes such immunological response is not seen and hence to cope up with the toxic response the macrophages seem to be playing great role in phagocytosis of damaged cells in the tissues/organs and thus increase in macrophages and basophails appears to be protective response during chromium exposure. Thus increase in macrophages in DLC can be used, as a diagnostic tool to assess the severity of the toxic effect.

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